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Carbocyclic **fatty** acids in plants: Biochemical and molecular genetic characterization of cyclopropane **fatty** acid synthesis of *Sterculia foetida*.

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ABSTRACT: **Fatty** acids containing three-member carbocyclic rings are found in bacteria and plants. Bacteria synthesize cyclopropane **fatty** acids (CPA-FAs) only by the addition of a methylene group from S-adenosylmethionine to the cis-double bond of monoenoic phospholipid-bound **fatty** acids. In plants CPA-FAs are usually minor components with cyclopropene **fatty** acids (CPE-FAs) more abundant. *Sterculia foetida* seed oil contains 65-78% CPE-FAs, principally sterculic acid. To address carbocyclic **fatty** acid synthesis in plants, a cDNA library was constructed from developing seeds during the period of maximum oil deposition. About 0.4% of 5,300 expressed sequence tags were derived from one **gene**, which shared similarities to the bacterial CPA-FA synthase. However, the predicted protein is twice as large as the bacterial homolog and represents a fusion of an FAD-containing oxidase at the N terminus and a **methyltransferase** at the C terminus. Functional analysis of the isolated full-length cDNA was conducted in tobacco suspension cells where its expression resulted in the accumulation of up to 6.2% dihydrosterculate of total **fatty** acids. In addition, the dihydrosterculate was specifically labeled by (methyl-14C)methionine and by (14C)oleic acid in the transgenic tobacco cells. In in vitro assay of *S. foetida* seed extracts, S-adenosylmethionine served as a methylene donor for the synthesis of dihydrosterculate from oleate. Dihydrosterculate accumulated largely in phosphatidylcholine in both systems. Together, a CPA-FA synthase was identified from *S. foetida*, and the pathway in higher plants that produce carbocyclic **fatty** acids was defined as by transfer of C1 units, most likely from S-adenosylmethionine to oleate.